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# FORUM

# Indirect Effects in Aquatic Ecotoxicology: Implications for Ecological Risk Assessment

**BENJAMIN L. PRESTON***
Pew Center on Global Climate Change
2101 Wilson Boulevard, Suite 550
Arlington, Virginia 22201, USA

ABSTRACT / Understanding toxicant effects at higher levels of biological organization continues to be a challenge in ecotoxicology and ecological risk assessment. This is due in part to a tradition in ecotoxicology of considering the direct effects of toxicants on a limited number of model test species. However, the indirect effects of toxicity may be a significant factor influencing the manner in which ecosystem structure and function respond to anthropogenic stressors. Subsequently, failure to incorporate indirect effects into risk assessment paradigms may be a significant source of uncertainty in risk estimates. The current paper addresses the importance of indirect effects in an ecotoxicological context. Laboratory, mesocosm, and whole ecosystem research into indirect effects is reviewed. The implications of indirect effects for ecological risk assessment and potential areas of profitable future research are also discussed.

Since its first use in the late 1970s, the term ecotoxicology has been defined generally as the study of the effects of anthropogenic toxicants on ecological systems (Truhaut 1977). This implies an emphasis on toxicant impacts on large-scale ecological phenomenon such as ecosystem structure (e.g., species abundance and diversity) and function (e.g., productivity and nutrient cycling). However, in practice, ecotoxicologists frequently focus on toxicity at smaller scales of biological organization, such as physiological mechanisms of toxicity and the responses of discrete endpoints in a single species to toxicant exposure (Clements and Kiffney 1994). This disparity represents a continuing fundamental challenge in ecotoxicology. Although there must necessarily be a connection between these two scales (Calow 1994), our ability to extrapolate from toxicity at the individual level to that at the ecosystem level is currently limited (Barnthouse and others 1987, Cairns and McCormick 1992, Chapman 1995, Newman 1995, Power and Adams 1997). As a result, ecotoxicologists find themselves in the precarious position of attempting to develop estimates of environmental risk sufficient to make informed management decisions with only a fraction of the information that is needed or at least a lack of certainty regarding what information is needed.

The source of this dilemma has frequently been attributed to the scientific methods that have traditionally been utilized in ecotoxicology, namely standardized single-species toxicity bioassays. Several authors have questioned the utility of such toxicity assays in understanding toxicant effects at the ecosystem level (Cairns 1983, 1989, Clements and Kiffney 1994, Chapman 1995), and there are certainly significant uncertainties associated with the ecological relevance of such data (US EPA 1998, Preston 2000). Toxicity assays are frequently conducted with a small body of test organisms, despite the significant difference in sensitivity that may exist among species and the diversity of species in natural ecosystems. There is a bias in toxicity databases for acute toxicity data, which is of questionable utility in predicting the consequences of sublethal exposures over extended time scales. Estimates of toxicity may also vary significantly with environmental conditions, confounding site-specific risk assessments (Spruage 1995, Preston and others 1999a, 2000). However, methods have been developed to address these challenges. Sublethal endpoints, including biomarkers of toxicity, have increased our ability to detect stress responses at low toxicant concentrations prior to impacts on ecosystem structure and function (Chen and others 1999; Karouna-Renier and Zehr, 1999; Hassanein and others 1999). Methods for adjusting toxicity estimates based upon environmental conditions such as pH and water hardness are commonly employed (US EPA 1986), and ecotoxicologists are developing a firmer understanding of interspecies variation in toxicant sensitivity (Versteeg and others 1999).

Despite these advances, a fundamental obstacle to risk assessment at higher levels of biological organization remains. That obstacle is the prevalent assumption that in natural environments, individual organisms function as discrete units, and thus, if one can rigor-

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**email:* prestonb@pewclimate.org

 

ously describe the effects of toxicants on individuals, extrapolations to the population, community, or ecosystem level can be performed readily. This assumption continues to be a foundation for ecotoxicology and ecological risk assessment, despite the existence of contradictory evidence. The biota of natural ecosystems are comprised of diverse populations of species interacting in complex ways both within and among populations. Such interactions are fundamental to the science of ecology and are known to have a great influence on the distribution and abundance of species (i.e., ecosystem structure) (Connell 1983, Schoener 1983, Williamson and others 1989, Rothhaupt 1990), which in turn drives ecosystem function. The strength of these interactions is such that it is not necessary for organisms to be directly exposed to toxicants to be affected by their release. Instead, a factor that is likely to be of great significance in how toxicants impact higher levels of biological organization is the way in which individuals and populations of organisms are affected indirectly through direct toxicant effects on other members of an ecosystem's biota with which they must interact.

The current paper reviews indirect effects and their significance in an ecotoxicological context. Published reports regarding laboratory, mesocosm, and whole-ecosystem studies pertinent to the understanding of indirect toxicant effects are discussed. The implications of indirect effects on the practice of ecological risk assessment are also addressed, and a number of future areas of research are suggested that may prove profitable in better understanding of indirect toxicant effects.

## Indirect Effects

The structure of biotic communities in natural ecosystems is a function of interactions between and/or among diverse species. This can be illustrated by examining the manner in which energy flows through an ecosystem's biota. Due to inefficiencies in energy transfer, the net energy available to the highest trophic level of an ecosystem may be orders of magnitude lower than that available to the lowest trophic level. However, as the highest and lowest trophic levels of an ecosystem rarely interact directly, the magnitude of the energy lost in transfer between these two extremes is dependent upon the number and diversity of intermediate trophic levels. This is the concept of indirect effects. Although populations are directly regulated through their interactions with other species, they may still be influenced indirectly by other populations with which they do not interact when effects are transduced through a series of intermediaries. A more general definition describes

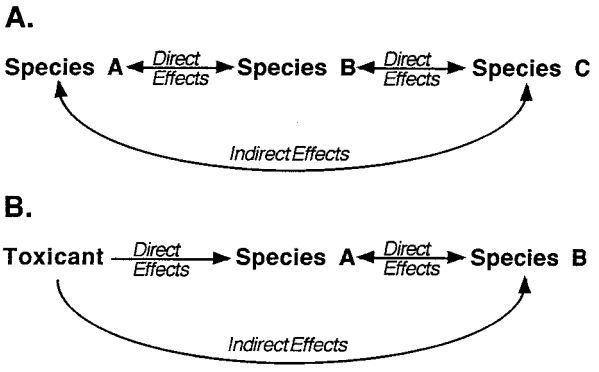


**Figure 1.** Conceptual model of indirect effects in (A) ecology and (B) ecotoxicology. Arrows indicate direction of effect. Indirect ecological effects may result from the interactions among interdependent species (A) or from the effects of toxicants on such interactions (B).

indirect effects as those by which the interactions between or among populations are affected by their interactions with other populations (Figure 1).

The principle ecological vehicles for indirect effects are interspecific interactions, particularly predation and competition, although other interactions such as mutualism, commensalism, or parasitism may also be important (Lafferty and Kuris 1999). For example, in freshwater systems, zooplanktivorous fish may influence phytoplankton abundance indirectly through their effects on zooplankton abundance (Polis and Winemiller 1996). Alternatively, specialized feeding on specific zooplankton species by predators may indirectly benefit populations of nonprey zooplankton species in competition with the prey. A wide variety of characteristics influence competition and predation between or among species (Atchison and others 1996). Often, interacting species have evolved together and thus may have become specialized to maximize niche exploitation and fitness. Predators may have specific search behaviors or feed selectively on vulnerable prey. Prey may alter habitat usage or develop other behavioral or morphological defenses to minimize predation risk. Life history characteristics such as growth or reproduction rate, clutch size, and parental investment may influence competitive interactions between or among species. Given that within any given trophic level there may be multiple competing species and that an individual species may prey upon or be preyed upon by multiple species, it is clear that indirect effects are frequent events that convey significant complexity to ecosystem structure. In addition, it has been suggested that indirect effects have similar or greater influence on species abundance as direct effects (Lampert and others 1989, Menge 1995).

Indirect effects carry a slightly different meaning in an ecotoxicological context (Figure 1). Toxicants, like species interactions, are capable of influencing species' distribution and abundance. Frequently, ecotoxicologists consider the direct effects of toxicity, such as adverse effects on survival, growth, or reproduction. However, species may also be affected indirectly through their interactions with other species that are directly affected by toxicity. Similar situations occur frequently in natural ecosystems in response to natural disturbance or catastrophe. For example, previously rare species may dominate following the disturbance or predators may exploit different prey as abundance and diversity in potential prey changes. One might expect ecosystems to respond in a like manner to toxicant release. Although a toxicant may directly affect species that are exposed or vulnerable to its method of action, the subsequent restructuring of the ecosystem's biota is likely to be a function of the subsequent indirect effects of toxicity on species interactions.

The nature of the indirect effects may be difficult to predict. Toxicant-induced suppression of competition or predation may have a beneficial effect for a particular species. However, reduction in a key prey resource may result in predator species decline despite resistance to the toxicant itself. Models of toxicity based upon the exposure of single species existing as isolated units are not sufficient to account for such complex ecological phenomenon. Although insightful research in indirect effects has been conducted in recent years by ecologists (Wootton 1994, Menge 1995), ecotoxicologists have largely ignored this phenomenon. It would seem that a better understanding of the significance of indirect effects in ecotoxicology would assist in the development of better conceptual models of toxicant effects on ecosystems and, subsequently, more ecologically relevant ecological risk assessments.

## Laboratory/Microcosm Studies

Laboratory studies of indirect effects have been limited, primarily due to the difficulties in reproducing higher levels of biological organization within the confines of a laboratory. However, a number of laboratory studies have contributed to understanding indirect effects through research in two areas: differential species sensitivity and predator-prey interactions.

### Differential Species Sensitivity

A fundamental consideration in toxicology is that different species possess different sensitivities to stress (Sprague 1995). As the structure of natural ecosystems is dependent upon the relative fitness of the individuals within the ecosystem, stressors that differentially alter fitness among individuals act as a novel selection factor that can potentially shift ecosystem structure to a different equilibrium. For example, whereas slow-growing, stress-tolerant species may have had poor fitness relative to faster-growing species before the toxicant release, afterwards their resistance conveys better relative fitness as the fast-growing species succumb to toxicant stress. Demonstrating the relative sensitivities of species assemblages to different toxicants may yield useful information regarding the responses of communities in natural ecosystems to toxicant exposure (Kooijman 1987).

Probabilistic modeling of species sensitivity distributions has been the most common method for comparing species sensitivities to toxicants (Kooijman 1987, Wagner and Lokke 1991, Aldenberg and Slob 1992, Okkerman and others 1993). Versteeg and others (1999) examined species sensitivity distributions for 11 different toxicants including heavy metals, surfactants, and pesticides. Sensitivity distributions were derived from single-species chronic toxicity assays. In all cases, species sensitivities spanned at least two orders of magnitude and in several cases four orders of magnitude. In addition, the relative sensitivity of tested species varied considerably among toxicants. For example, the zooplankton *Brachionus, Daphnia*, and *Ceriodaphnia* were among the most sensitive to the surfactant dodecyl linear alkylbenzene sulfonate ($C_{12}$LAS) and copper, but least sensitive to lindane. For the toxicants cadmium, phenol, and 3,4-dichloroaniline (3,4 DCA), *Daphnia* and *Brachionus* appeared at opposite ends of the distribution with *Brachionus* being less sensitive and *Daphnia* more sensitive. McDaniel and Snell (1999) used a similar approach to look at sensitivity distributions among nine species of rotifer in response to cadmium and pentachlorophenol (PCP) exposure. Sensitivities spanned two orders of magnitude for both toxicants, despite the taxonomic relatedness of the species. Just as above, relative sensitivity among species varied with the toxicant as well as the end point (i.e., 24-hr mortality or 30-min in vivo esterase activity).

Methods such as these have been applied in ecological risk assessments of atrazine, cadmium, copper, and tributyltin to estimate ecosystem threshold toxicity levels (Solomon and others 1996, Hall and others 1998, Cardwell and others 1999). The advantage of this approach is that one does not have to assume that one species is sufficiently sensitive or representative of a community of organisms. Species sensitivity distributions may have other applications as well. For example, one can predict how assemblages respond differently to different toxicants, and, perhaps more importantly,

one may predict which communities (i.e., phytoplankton, zooplankton, planktivorous, or piscivorous fish) within an ecosystem will be most severely impacted. Subsequently, one may be able to deduce how species interactions will be altered after exposure. The results from such an analysis have been demonstrated to yield estimates of toxicity thresholds comparable to those of mesocosm studies where assemblages of species are tested simultaneously (Versteeg and others 1999).

Such approaches do have limitations, however. First, the individual species within a selected assemblage are tested in isolation rather than together as they would be found in nature. Reynoldson and others (1994) observed that toxicity end points for single species tested in isolation differed from those for species tested in the presence of other indigenous species. Second, the end points used to create the distributions may not be accurate representations of toxicity thresholds as they may be comprised of acute end points or the end points may be calculated using hypothesis testing (e.g., NOEC and LOEC) (Crane and Newman, 2000). Third, the utility of sensitivity distributions is dependent upon the number of species for which data are available (Roman and others 1999, Newman and others 2000). The more representative the assemblage is of those in natural ecosystems, the more ecologically relevant the analysis.

### Predator–Prey Interactions

As mentioned previously, direct assessment of species interactions in the laboratory is a difficult task as it necessitates testing two or more species simultaneously. Nevertheless, a number of laboratory studies have described the effects of toxicants on predator–prey interactions among aquatic organisms. Such interactions may be complex, depending on both the morphology and various behaviors of predator and prey (Atchison and others 1996), providing various pathways for toxicants to disrupt the interaction. Such disruption may have important implications for both species abundance as well as the bioaccumulation of toxicants through food chains. A number of methods have been used to observe predator–prey interactions in microcosms including video imaging of the interaction (Gomez and others 1997, Preston and others 1999b), examination of predator stomach contents (Clements and others 1989), or monitoring changes in predator and/or prey population density (Hatakeyama and Yasuno 1987, Sarma and others 1998).

Zooplankton grazing upon phytoplankton has been used as an indicator of toxicity for two decades (Geiger and Buikema 1981, Kersting and Van der Honing 1981, Ullrich and Millemann 1983). Janssen (1992) calculated the filtration and algae ingestion rates of the rotifer *Brachionus calyciflorus* in response to sublethal concentrations of copper, PCP, 3,4-DCA, and lindane (see also Ferrando and Andreu-Moliner 1993, Ferrando and others 1993). Adverse effects were observed within 5 hours of exposure. Juchelka and Snell (1994) subsequently used fluorescently labeled latex microspheres in place of algae to test the feeding behavior of *B. calyciflorus* in response to a number of toxicants including metals, pesticides, and solvents. This methodology also has been used to examine toxicant effects on feeding in cladocerans and ciliates (Juchelka and Snell 1995). Ingestion end points are often more sensitive to toxicity than survival (Juchelka and Snell 1994, 1995).

A number of studies have been conducted on predator–prey interactions between invertebrate prey and predators as well invertebrate prey and vertebrate predators. Clements and others (1989) observed that caddisfly (*Hydropsyche morosa*) predation by stoneflies (*Paragnetina media*) in experimental microcosms increased significantly after sublethal exposure to copper, presumably due to toxicant-induced changes in predator avoidance behavior of *H. morosa*. These effects contradicted prior reports of heavy metal resistance in caddisflies (Clements and others 1988). Ham and others (1995) reported that predation of the isopod crustacean (*Asellus aquaticus*) by the turbellarian (*Dendrocoelum lacteum*) decreased in response to sublethal cadmium exposure. In addition, although *D. lacteum* was over an order of magnitude less sensitive to cadmium than *A. aquaticus*, it was proposed that cadmium-induced reduction in the prey population would have adverse effects on reproduction and competitive ability of *D. lacteum* even when not directly affected by toxicity. Gomez and others (1997) examined the effects of sublethal PCP exposure on the predator–prey interaction between *B. calyciflorus* and the predatory rotifer *Asplanchna girodi*. PCP reduced *A. girodi* capture and ingestion efficiency. Preston and others (1999b) studied the effects of PCP on the vulnerability of several species of rotifer to predation by *A. girodi*. Due to differential responses of prey to PCP, changes in prey vulnerability after exposure varied among prey species. Tagatz (1976) observed that mirex increased swimming activity of grass shrimp (*P. vulgaris*) and pinfish (*Lagodon rhomboides*), causing increased visibility to predators. Similarly, Weis and Weis (1995) reported that increased swimming among mummichog (*Fundulus heteroclitus*) larvae exposed as embryos to methylmercury increased predation by grass shrimp (*Palaemonetes pugio*) and cannibalism by adult mummichogs. PCP has been demonstrated to increase the swimming activity of *B. calyciflorus* (Janssen and others 1994, Preston and others

1999c) and inhibit the predator avoidance behavior of juvenile guppies (*Poecilia reticulata*) (Brown and others 1985).

Predator–prey interactions are also the primary mechanism underlying bioaccumulation of toxicants through food chains. Toxicants may have adverse effects on predators that are not affected by nominal concentrations through the accumulation of toxic levels of contaminants through ingestion of contaminated prey. For example, reproduction of the predatory rotifer *Asplanchna sieboldi* was reduced significantly when fed *B. calyciflorus* that had been exposed to methyl parathion, despite the fact that *A. sieboldi* was not exposed directly to the toxicant (Sarma and others 1998). Similarly, reproduction of the guppy (*P. reticulata*) was impaired when fed midge (*Chironomus yoshimatsui*) larvae exposed to cadmium (Hatakeyama and Yasuno 1987). However, adverse effects were not apparent until after 30 days of experimentation. This demonstrates that adverse indirect effects may be absent over short time scales, but significant over longer time scales.

One persistent limitation of studies directed at toxicant effects on predator–prey interactions is that the interaction is commonly used as an indicator of toxicity. Although speculation is frequently offered regarding the broader ecological implications for a population or community, little attempt has been made to quantitatively assess impacts at higher levels of biological organization sufficient to be of use to risk assessors. Preston and others (1999c), used mathematical models to estimate the effects of toxicant-induced changes in the swimming behavior of *B. calyciflorus* on the risk of ingestion by *A. girodi* over the course of its estimated lifespan. Such an approach allows one to use laboratory data to make ecologically relevant predictions regarding the fate of natural populations. More recently, Preston and Snell (2000) used a similar approach to compare the relative effects of toxicant-induced changes in predation risk versus toxicant-induced changes in reproduction for several species of rotifer exposed to sublethal PCP concentrations. Results indicated that toxicant effects on predator–prey interactions were negligible when compared to toxicant effects on reproduction, suggesting single-species toxicity data at the population level may be useful in examining toxicant effects on species interactions. Such studies demonstrate that relatively simple laboratory tests may provide useful information on higher levels of biological organization if the results are placed in a suitable ecological context.

## Mesocosm Studies

Despite the mechanistic data that may be gained through small-scale experimental designs, their predictive power remains limited. Natural ecosystems clearly possess significantly greater complexity, and thus more complex assessment tools are necessary to simulate toxicant impacts at higher levels of biological organization with an appreciable degree of realism. To date, the most valuable data regarding indirect toxicant effects have come from experimental mesocosms consisting of in situ enclosures where multiple species and trophic levels are allowed to react to toxicant exposures while interacting with one another. Such mesocosms have the advantage of testing toxicant effects over long periods of time, under natural environmental conditions, utilizing indigenous species that have a history of interacting under site-specific conditions.

Day and others (1987) tested the effects of the pesticide, fenvalerate, on plankton communities in lake enclosures. The direct effects of fenvalerate caused inhibition of zooplankton feeding behavior and a reduction in size of most large-body zooplankton (e.g., cladocerans and copepods) populations. However, small-bodied rotifer populations experienced a dramatic increase in size due to their resistance to fenvalerate relative to other zooplankton, which caused a reduction in competition. Kasai and Hanazato (1995) investigated the effects of the herbicide, simetryn, on plankton communities. Simetryn directly reduced phytoplankton abundance. In addition, a number of indirect effects were observed, including decreased dissolved oxygen concentrations, increased dissolved nutrient concentrations, changes in phytoplankton species succession, and reduction in zooplankton abundance. Boyle and others (1996) observed that the pesticide diflubenzuron caused direct toxicity to zooplankton, with residual indirect effects causing increased algal abundance (due to reduced zooplankton grazing) and reductions in bluegill populations (due to reduced food availability for juvenile fish). Differential sensitivity among zooplankton also resulted in the dominance of relatively few species compared to control conditions. Peither and others (1996) observed direct adverse effects of lindane on insects and copepods, while cladocerans and rotifers were not directly affected. However, due to the loss of the predatory insect *Chaoborus flavicans*, the predatory rotifer *Asplanchna priodonta* experienced competitive release leading to a reduction in the population size of its prey, *Keratella quadrata*. In contrast, Sierszen and Lozano (1998) observed that within a freshwater zooplankton community, azinphos-methyl had adverse effects on cladocer-

ans while copepods and rotifers were unaffected. Lastly, Faber and others (1998) reported reductions in net zooplankton abundance in response to glufosinate ammonium and bialaphos, but the dominant taxa switched from copepods before exposure to rotifers after exposure.

Similar studies have been conducted using stream mesocosms. Hansen and Garton (1982) studied the effects of the herbicide diflubenzuron on natural biota introduced into a laboratory stream. Diflubenzuron exposure directly reduced the density of the insect community, causing indirect effects on the abundance of algae and fungi. In addition, order of magnitude differences in sensitivity were observed among insect species, resulting in a reduction in community diversity as more sensitive species were eliminated. Similarly, Clements and others (1988) observed that copper reduced the net size and diversity of the insect community in a laboratory stream and differential sensitivity among species led to variation in species dominance among copper treatments. Ward and others (1995) reported the effects of chlorpyrifos on the biota of outdoor artificial streams. Chlorpyrifos exposure reduced the abundance of 21 of 55 invertebrate taxa, with indirect effects on periphyton abundance and organic matter within the stream.

These mesocosm studies demonstrate that in addition to their direct effects, toxicants can cause indirect effects within or among trophic levels. The most common indirect effects appear to be release of tolerant/resistant species from competition and/or predation, resulting in a shift in ecosystem structure. However, as Boyle and others (1996) observed, population declines may occur even in tolerant species if their food supply is reduced. These effects are readily observable in mesocosms, but cannot be predicted from single-species assays. The sensitivity of mesocosms to toxicity relative to single-species assays is also an important consideration. For example, Lampert and others (1989) reported that *Daphnia* exposed to atrazine in a food chain experienced adverse effects at lower concentrations than when *Daphnia* was tested in isolation. This was attributed to atrazine-induced reduction in *Daphnia's* phytoplankton food source. Depending upon the interactions within an ecosystem, individual species may be adversely affected by toxicants at concentrations lower than can be predicted by single-species assays. Thus, placing single-species toxicity data in an ecologically relevant context requires a priori knowledge regarding the ecology of the test species and the relative sensitivities of other species with which it may directly or indirectly interact. Another consideration is the response of ecosystems to multiple stressors. Given that the relative sensitivity of species to toxicants may vary among toxicants (Versteeg and others 1999), it may be difficult to predict an ecosystem's response to multiple toxicants from single-species data, particularly if there is an interaction between or among toxicants. However, Hoagland and others (1993), did not observe a synergistic interaction between atrazine and bifenthrin on the biota of mesocosm communities, presumably because the toxicant present in highest concentration masked the effects of the other.

## Whole Ecosystem Studies

If ecotoxicologists are to understand toxicant impacts at high levels of biological organization, then ideally the experimental scale should target whole ecosystems (Frost and others 1988, Schindler 1990). Unfortunately, there are obvious obstacles to conducting ecotoxicological experiments at the level of a whole ecosystem. First, experimentally exposing an entire aquatic ecosystem to a toxicant contradicts the goals of ecotoxicology. Second, the effort required to quantify the structure and function of an ecosystem over ecologically relevant time scales is often prohibitive. Although ecological risk assessments are routinely performed over large geographic areas (Hall and others 1998), such assessments are often conducted retrospectively, and thus there may not be historical preimpact reference data against which to compare current data.

As a result, the most relevant data for indirect effects of environmental stressors on whole ecosystems come from whole-lake acidification experiments. The most famous of such experiments is that of Little Rock Lake, Wisconsin, USA, where one of two lake basins was experimentally maintained at reduced pH between 4.7 and 5.6 over five years (Brezonik and others 1986, Watras and Frost 1989). The other basin was separated by a vinyl curtain and used as a reference site with a mean pH of approximately 6.1. Webster and others (1992) reported a variety of direct and indirect effects on the biota of Little Rock Lake following acidification. Immediate population declines were observed for the cladoceran *Daphnia dubia* and the mayfly *Leptophlebia* sp., as well as several species of invertebrate predators. Acidification also led to the proliferation of benthic filamentous algae, which led to indirect increases in benthic cladocerans (*Chydorus*) and caddislies (*Oxyethira*) and provided increased refuge for juvenile yellow perch. Gonzalez and Frost (1994) studied the effects of acidification on rotifer species in Little Rock Lake and compared those effects to predictions based upon laboratory responses of individual species to pH changes. In laboratory bioassays, when food was lim-

ited, *Keratella cochlearis* experienced reduced survivorship and reproduction, while *Keratella taurocehpala* was unaffected. In contrast, acidification of Little Rock Lake resulted in a decrease in food availability for both species. Subsequently, *K. cochelaris* experienced sharp declines in abundance while *K. taurocephala* experienced an increase in abundance due to the reduction in the abundance of invertebrate predators.

Whole-lake acidification experiments have also provided insight into the persistence of effects associated with ecosystem stress. In a study of whole-lake acidification in Ontario, Canada, Havas and others (1995) observed that the recovery of the biota of an acidified lake was characterized by the dominance of a few low pH-tolerant species. However, the work of Frost and others (1998) at Little Rock Lake indicated that ecosystem community structure eventually resembled that of the preacidification community, suggesting the increased fitness of low pH-tolerant species declines when the stress is removed and full biotic recovery may be achieved. In addition, the exposure of an ecosystem to a single stressor may promote a variety of secondary stressors that may interact to affect the biota. Frost and others (1999) reported that the acidification of Little Rock Lake led to an increase in the penetration of ultraviolet radiation (UVR) into surface waters. Similarly, Williamson and others (1999) reported that lake acidification increased UVR stress on zooplankton community structure by reducing dissolved organic carbon. Thus, the complexity of ecosystems may impede attempts to develop uniform theory regarding their responses to stressors, mandating consideration of site-specific characteristics.

## Implications for Ecological Risk Assessment

Upon review of the literature, it becomes apparent that the manner in which organisms respond to stressors in their environment may differ significantly from what would be expected based upon single-species laboratory tests. This disparity occurs predominantly because the fitness of organisms in natural ecosystems is dependent upon their interactions with other indigenous species, and failure to consider such interactions prevents meaningful predictions regarding responses to stress. Thus, conducting an ecological risk assessment requires an appreciation for the complexity of natural ecosystems, an understanding of ecosystem structure and the functionality of the subunits within that structure, and the realization that there may be multiple mechanisms driving an ecosystem's response to stress.

More specifically, one of the critical implications of indirect effects for ecological risk assessment is that adverse effects can occur in populations that are not directly exposed or affected by the toxicant(s) of concern. This phenomenon of "action at a distance" (Spromberg and others 1998) means that if risk assessors are to make ecologically relevant predictions of risk to a single or selected assemblage of species, consideration of those species' individual tolerances and/or responses to toxicants may not be sufficient (Lampert and others 1989). This leaves risk assessors with two options. First, a more detailed study of the ecology of the species of interest can be performed to identify interspecific interactions that influence that species' distribution, abundance, and fitness. Subsequently, ecotoxicological assessments can be conducted to determine the extent to which such interactions may be impacted by toxicant exposure. However, as indirect effects can be transduced through an extensive chain of intermediaries, the complexity of such an approach may be prohibitive. Second, risk can be assessed not to single species but rather to the biota of an ecosystem as a whole using probabilistic methods involving species sensitivity distributions as previously discussed. Again, the rigor of such an approach is dependent upon the quality of the data utilized [i.e., number of species, end point(s) used]. However, if a toxicity threshold can be calculated for the collective biota of an ecosystem, then presumably that threshold will be applicable to all species and their interactions (Preston and Snell 2000). Maintenance of environmental toxicant concentrations below such a threshold would protect against direct and indirect effects.

Another implication of action at a distance involves the use of reference sites in ecological risk assessment. Reference sites are frequently used to compare the distribution and abundance of biota in an impacted site to that of a pristine or nonimpacted site. Often these sites may be located in close geographic proximity to the impacted site in an attempt to match abiotic and biotic characteristics between the two. However, the appropriateness of a site as a reference may be compromised by indirect effects. Spromberg and others (1998) used a modeling approach to demonstrate that uncontaminated sites may be affected indirectly by contaminated sites through the migration of biota. For example, a contaminated site could be a small patch in the relatively large range of an animal population. As a result, periodic foraging by that population within the contaminated site may have adverse indirect effects over a much larger geographical area. Thus adjacent ecosystems, although not directly contaminated, may be still be affected at a distance by contamination and thus inappropriate as reference sites.

Furthermore, there is an assumption in ecological risk assessment that large population size or density is indicative of ecosystem health. However, a number of examples have been presented where increases in the population size of certain species occurred as a result of adverse ecological impacts. Thus, reduced population size of a species of concern at a contaminated site relative to a population at a reference site is not necessarily indicative of adverse effects of contamination on the population in the former. Instead, arguments can be made that such observations could be indicative of ecological impacts at the reference site as well. Thus, comparisons among multiple populations, preferably using multiple reference sites, are necessary to ensure the health and suitability of a particular reference site and that conclusions regarding effects at the site of interest are reasonable.

## Future Needs

There is a clear need to increase the ability of ecotoxicologists to understand toxicant impacts at higher levels of biological organization. However, this may be achieved through a number of different methods. As discussed by Schindler (1996), it is unlikely that complex, long-term ecosystem level toxicity assessments will become routine in ecotoxicology. However, the utility of the Little Rock Lake acidification experiment demonstrates that such large-scale studies may be of great benefit. For example, the results from more common small-scale bioassays or mesocosm experiments could be calibrated against whole-ecosystem data to determine their ecological relevance (Schindler 1996). In addition, Cottingham and Carpenter (1998) reported that indices of ecosystem function were better indicators of change in a lake ecosystem in response to experimental nutrient enrichment than data for individual species. Ecotoxicologists may overcome some of the current limitations regarding the detection of ecological effects by shifting their focus from the biotic to the abiotic environment, which may be easier to quantify over large temporal and spatial scales. A small number of whole-ecosystem experiments might serve as useful classrooms for ecotoxicologists and assist in integrating a ecological theory into ecotoxicology (Clements and Kiffney 1994, Schindler 1996).

Another important need for ecotoxicologists is to develop a better understanding of the natural variation in the distribution and abundance of organisms that occur in natural ecosystems. Laboratory variation in toxicity assays has been a critical concern for many years, yet variation in populations of organisms in the wild has largely been ignored by ecotoxicologists. Without quantifying seasonal and yearly variation in natural population, it may be difficult to assign cause to observed changes in an ecosystem's biota. For example, Cottingham and Carpenter (1998) reported that the natural variation in individual species in a lake ecosystem was so great that they could not find significant differences in populations from an acidified and reference site. Furthermore, the emphasis on hypothesis testing in ecotoxicology has led to an overemphasis on statistically significant effects. There may be a great disparity between what is statistically and ecologically significant (Calow 1994, Crane and Newman 2000). For example, the effective concentration 20% ($EC_{20}$) has been proposed as a toxicity benchmark in ecological risk assessment due to the assumption that a 20% change is the minimal detectable difference in population characteristics in the field (Suter 1996). Yet a 20% effect in some aspect of a species' life history could have a significant impact on its long-term sustainability (Snell and Serra 2000). What ecotoxicologists can readily detect should not be the only rubric against which assessments of anthropogenic impacts to ecosystems are judged, as convenient end points may not always be sufficient to detect effects of interest (Chapman 1995, Cottingham and Carpenter, 1998).

In light of this, it may be helpful for ecotoxicologists to become familiar with other possible end points that may be better indicators of ecosystem-level effects. For example, ecotoxicological studies at the population level are relatively easy to conduct in both the laboratory and field. Snell (2000) found that the 28-day carrying capacity of the rotifer *Brachionus calyciflorus* was approximately an order of magnitude more sensitive to PCP than other sublethal end points. A number of authors have commented on the utility of indices of population growth such as the intrinsic rate of increase ($r$) in ecotoxicological assessments (Snell and Moffat 1992; Walthall and Stark 1997, Forbes and Calow 1999). Population measures can also be readily incorporated into ecological models, greatly enhancing the information that can be gained from laboratory and field data. Snell and Serra (2000) used toxicant effects on $r$ from laboratory tests to model the effects of reproductive toxicity in *Asplanchna* on the probability of extinction. Preston and Snell (2000) used similar data to model toxicant effects on short-term population dynamics in rotifer species. Several authors have also proposed the use of life-table analysis to estimate toxicant effects (Newman 1995, Grant 1998, Bechmann 1999, Roex and others 2000). The use of population genetics has also been applied in ecotoxicological studies (Fore and others 1995a, b, Mulvey and others 1995, Schlueter 1995, Newman and Jagoe 1998).

In addition, ecotoxicologists need to become more proficient with tools for making measurements at the community level. A number of quantitative methods currently exist that are routinely used by ecologists (Newman 1995). Methods for investigating toxicant effects on predator–prey interactions, including predation modeling, have been described previously. In addition, Sandheinrich and Atchison (1990) used an optimal foraging model to predict changes in bluegill diet in response to toxicant stress. Studies of toxicant effects on species competition also can be conducted in the laboratory by examining population dynamics of species exposed to toxicants and competitors simultaneously. Alternatively, modeling approaches can be utilized for studying competitive interactions (Newman 1995). Toxicant effects on ecosystem structure can be quantified through various means including measuring species richness (number of species) and species diversity (equatibility in population sizes among species), data that are routinely collected in ecological monitoring (Newman 1995). A wide variety of functional aspects of an ecosystem also can be studied including photosynthesis and respiration, chlorophyll concentration, sedimentation, nitrogen fixation, decomposition, and nutrient cycling (Newman, 1995).

Another issue that has yet to be satisfactorily decided is that of functional redundancy (Pratt and Cairns 1996), which assumes that individuals and species are fungible, and the losses sustained by one species will be balanced by the gains in another species, provided the latter occupies a similar functional niche within the ecosystem (Walker 1991, Lawton and Brown 1993). Thus, in an ecosystem with high functional redundancy, there is an inherent capacity to absorb stress while maintaining function. As discussed previously, a number of studies have observed increases in the abundance of certain species due to population declines of their competitors. For example, Frost and others (1995) observed that zooplankton biomass during the Little Rock Lake acidification experiment remained at high levels, despite the loss of individual species. An alternate theory is the "rivet popper hypothesis," in which loss of individuals and species leads to increased instability in the ecosystem and reduced resilience to future stress (Ehrlich and Ehrlich 1981). Belovsky and others (1999) reported that variability in population size and environmental conditions was an important factor determining the probability of population extinction. However, different trophic levels of an ecosystem may possess different degrees of redundancy. Species richness may be quite high among primary producers, while the top-level predators may consist of only a few species (Schindler 1996). The loss of top-level predators may have significant destabilizing indirect effects throughout the ecosystem (Paine 1966, Carpenter and others 1985, Power 1990). Thus, an ecosystem's ability to absorb stress is dependent upon which aspects of an ecosystem's structure and function are targets of the stressor (Cairns and Pratt 1986). As an ecosystem's ability to absorb and/or recover from stress is an important consideration in predicting both short and long-term ecosystem integrity and sustainability, further clarification of the significance of functional redundancy in ecotoxicology seems prudent.

It must be accepted, however, that despite any increased attempts to incorporate ecological theory into ecotoxicology, current methodologies, including single-species toxicity assays, will continue to be the dominant tool in ecotoxicological assessments. A number of steps can be taken to maximize the utility of such data. For example, the bias in toxicity databases for acute data is of limited use to ecotoxicologists and risk assessors for anything other than comparing the relative toxicity of different chemicals. Although species loss is certainly an ecologically relevant effect, a number of effects can cause species loss at sublethal toxicant concentrations (Snell and Serra 2000). Although numerous methods for extrapolating chronic end points from acute data have been presented (Mount and Stephan 1967, Kenaga 1982, Sun et al., 1995), such extrapolations cannot be as reliable as the data generated from chronic toxicity tests themselves and frequently add uncertainty to a toxicity assessment. There is also a critical need for greater diversity in test species and end points. Ecotoxicologists should not assume that a certain end point or species is the most representative and/or sensitive without first establishing a basis for comparison (McDaniel and Snell 1999, Snell 2000). Diversification in toxicity databases will allow ecotoxicologists to replace hypothesis testing (i.e., NOEC/LOEC) as a means for establishing toxicity thresholds with more rigorous, probabilistic methods, thereby reducing uncertainty in ecological risk assessments (Wagner and Lokke 1991, Okkerman and others 1993, Crane and Newman 2000).

## Conclusions

Cairns (1990) argued that the discipline of ecotoxicology has been driven largely by a tradition of methodology and ideology rather than sound scientific principles. There has been a clear reluctance in ecotoxicology to address ecosystem complexity through experimentation. This can be attributed to at least two reasons. First, there is a common, yet mistaken, assumption that large-scale phenomenon are too complex to

study rigorously (Newman 1995). Second, environmental managers and risk assessors need tools and information that can be applied readily to a decision-making process in a timely manner, resulting in a bias for assessment tools that target low levels of biological organization that can generate rapid results. However, if ecotoxicology is to advance as a science, then it must behave as a science in practice (Newman 1996). If ecotoxicologists continue to rely upon overly simplified conceptual models of ecosystem structure and function, little progress can be made in understanding toxicant effects at the ecosystem level. Indirect effects are a major consideration in ecology, and therefore, they must also be a consideration in ecotoxicology and ecological risk assessment. Future experimental ecotoxicology could be profitably directed at characterizing common indirect effects resulting from ecosystem disturbances and quantifying the relative impact of direct and indirect effects of toxicity on ecosystems to validate and/or improve current risk assessment methodologies.

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